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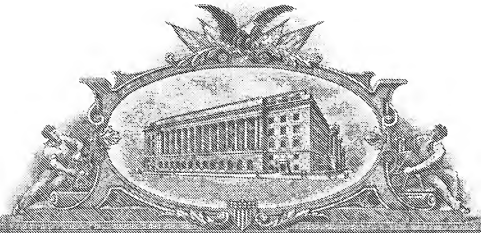

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APPLICATION NUMBER: ***60/543,986***
FILING DATE: ***February 12, 2004***
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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INVENTOR(S)

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☒ *Additional inventors are being named on the* 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Technetium- and Rhenium-Bis(heteroaryl) Complexes, and Methods of Use Thereof

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 16 ☐ CD(s), Number ____

☐ Drawing(s) *Number of Sheets* ____ ☒ Other (specify) Return-Receipt Postcard

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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[Page 1 of 2]

Respectfully submitted,
SIGNATURE Dana Dordon
Date February 12, 2004
REGISTRATION NO. (*if appropriate*) 44,719
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Docket Number BSA-014.61

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Attorney Docket No.: BSA-014.61

PROVISIONAL APPLICATION FOR PATENT

Technetium- and Rhenium-Bis(heteroaryl) Complexes, and Methods of Use Thereof

Uniting Fluoresence and Radioimaging. Synthesis, Bioconjugation and Spectral Characterization of Re and ^{99m}Tc Complexes of a Single Ligand for Both Fluoresence and Radioimaging Studies.

Two of the most widely employed techniques for visualizing specific biological processes are fluorescence microscopy and radioimaging. Fluorescence microscopy is a powerful tool for looking at the distribution of fluorescent probes in vitro and for studying the dynamics of protein motion. The high spatial resolution of this method allows for accurate localization of the probe within a cell. Radioimaging on the other hand allows for the study of molecular processes in vivo by detecting the distribution of a molecule tagged with a radionuclide. Functional radioimaging studies can be carried out in humans or in animals non-invasively, which in the latter case, are now often performed on commercially available scanners that are designed specifically for producing high resolution images (mm scale) of animals.

There have been an increasing number of reports where compounds used to target radionuclides to specific receptors for in vivo imaging studies, have also been labeled with fluorescent probes so that the localization of the bioconjugate can be determined at the cellular level, which is beyond the resolution of radioimaging techniques like positron emission tomography (PET) and single photon emission computed tomography (SPECT). Gallazzi, F.; Wang, Y.; Jia, F.; Shenoy, N.; Landon, L.A.; Hannink, M.; Lever, S.Z.; Lewis, M.R. *Bioconjugate Chem.* **2004**; and (b) Bullok, K.E.; Dyszlewski, M.; Prior, J.L.; Pica, C.M.; Sharma, V.; Piwnica-Worms, D. *Bioconjugate Chem.* **2002**, *12*, 1226.

Unfortunately, the structures of common fluorescent probes and radionuclide prosthetic groups are significantly different which introduces a potential source of error when comparing data from in vitro and in vivo experiments. The optimal system would be one in which the fluorescent and radioactive prosthetic groups are iso-structural.

Technetium-99m is the most widely used radionuclide in diagnostic medicine owing to its ideal nuclear properties, low cost and widespread availability. There are a significant number of different Tc complexes that are used clinically including agents that are designed to image bone metastases and myocardial function. Re(I) complexes on the other hand, have been used to prepare luminescent probes. These complexes are particularly useful for studying biological processes in vitro because of their long-lifetime, polarized emission and large Stoke's shift which overcomes issues of self-quenching. Based on the fact that the coordination chemistry of the two congeners is very similar, it should be possible to design a ligand that forms a fluorescent Re complex and a stable ^{99m}Tc complex. Such a system, which should also possess the ability to be linked to a targeting agent, would allow images obtained on a fluorescent microscope to be directly correlated with in vivo imaging studies.

Recently we reported the synthesis of a Tc(I) binding ligand which was referred to as a single amino acid chelate (SAAC) (**1**, **Figure 1**). The SAAC forms an inert complex with the $M(CO)_3^+$ core (M = Re, ^{99m}Tc) and it can be incorporated into peptides as if it were a natural amino acid. To prepare a SAAC type ligand whose Re complex is fluorescent, while retaining the ability to bind ^{99m}Tc, N-α-Fmoc-L-lysine was reacted with quinoline aldehyde in the presence of $Na(OAc)_3BH$ to give the bifunctional ligand **3** (**Scheme 1**). The desired product, which can be produced in multi-gram quantities, was

isolated in excellent yield following column chromatography. The Re complex **4a** was synthesized by reacting **3** with $[NEt_4]_2[Re(CO)_3Br_3]$. The complex was isolated as the TEA salt by column chromatography.

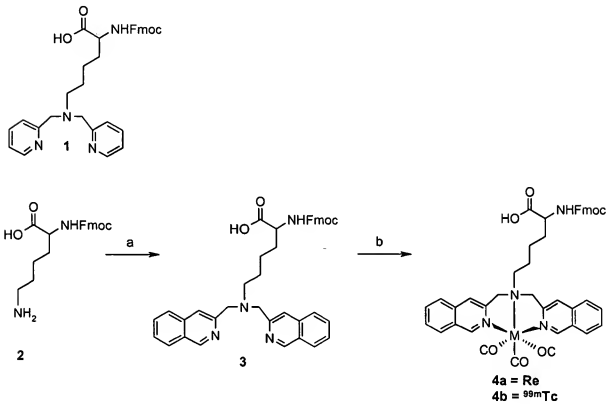


Figure 2 shows the absorbance spectrum of compound **4a** from 250 to 700 nm in 5% chloroform 95% ethylene glycol. The rhenium compound has appreciable absorbance in the UV and blue regions of the UV-visible spectrum with a peak absorbance at 301 nm. The extinction coefficients for compound **4a** were 13,200 $M^{-1}cm^{-1}$ at 301 nm and 2250 $M^{-1}cm^{-1}$ at 366 nm. Emission was monitored from 400 to 700 nm with excitation wavelength of 366 nm in 1 nm increments with an integration time of 0.25 sec and bandwidths of 5 nm. Spectra were acquired in ethylene glycol and chloroform solutions both in the presence of air and under nitrogen (**Figure 3**). The rhenium probe has two distinct transitions giving rise to peak fluorescence intensities at 425 and 580 nm.

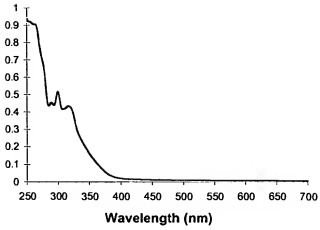


Figure 2: Absorbance spectra for compound **4a** (40 μM solution in 5% chloroform 95% ethylene glycol).

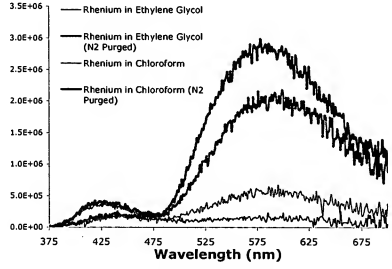


Figure 3: Emission spectra for compound **4a** in 100% ethylene glycol (under nitrogen and air) or chloroform (under nitrogen or air).

To assess whether the rhenium probe may be suitable for polarization based fluorescence assays, the steady state fluorescence anisotropy was measured as a function of excitation wavelength (**Figure 4**). A solution of compound **4a** was prepared in 100% ethylene glycol and cooled to – 20 °C to slow the rotational movement of the fluorescent

molecule. Under these conditions the fluorescence anisotropy increases to a limiting anisotropy of 0.35 at a wavelength of 424 nm.

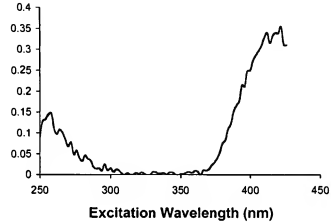


Figure 4: Steady state anisotropy as a function of excitation wavelength. Excitation was collected in 2 nm increments with an emission wavelength of 590 nm and an integration time of 3 sec, and bandpasses of 5 nm.

Compound **4a** has a number of attractive properties as a ligand for luminescence studies. The probe absorbs in the ultraviolet region spectrum, and has an emission maximum at approximately 585 nm which avoids complications that arise in physiological studies due to cell auto-fluorescence. In similar respects, the probe has an extremely long lifetime ranging between 1 to 16 μs. This is also beneficial for physiological studies because cell auto-fluorescence occurs on the nanosecond time scale, and can therefore be eliminated using time-gating techniques so long as the probe under investigation has a sufficiently long lifetime. An additional advantage to compound **4a** is that it exhibits fluorescence anisotropy making it useful for small molecule - cell receptor or protein-protein binding studies in which the rotational mobility of the probe can be

monitored as a function of binding. The one drawback to compound **4a** is that it has a low quantum yield, which ranges from 0.0027 in chloroform in the presence of air to 0.0145 in ethylene glycol under nitrogen.

To determine if the SAACQ ligand forms a stable ^{99m}Tc complex, compound **3** was added to $[Tc(CO)_3(OH_2)_3]^+$, which was prepared by adding TcO_4^- to a commercially available carbonyl labeling kit. The desired product **4b** was isolated in high yield even when very small amounts of the ligand were used. The stability of the complex to transchelation was investigated by incubating two separate samples of **4b** with 1000 fold excess of cysteine and histidine in PBS buffer heated to 37°C. After 24 hours there was almost no sign of degradation which clearly indicates that compound **4b** is suitably robust for use in vivo.

Because the SAACQ ligand and the SAACQ-Re complex are amino acid analogues they can be readily incorporated into a peptide at any position using a conventional peptide synthesizer. To demonstrate this feature the SAACQ and SAACQ-Re complexes were incorporated within fMLF; a targeting sequence which has been used to guide radionuclides to the formyl peptide receptor as a means of imaging sites of infection and inflammation. The peptides fMLF(SAACQ)Gly (**5**) and fMLF[(SAACQ-$Re(CO)_3)^+$]G (**6**) were prepared following standard FMOC synthetic methods using a glycine loaded SASRIN resin and HBTU-HOBt as the coupling agent. The peptides were isolated using a standard cleavage cocktail (94% TFA, 2% EDT, 2% TIS and 2% water) and the products purified by HPLC. The HPLC purification was needed as a result of epimerization of the methionine residue which is known to occur under normal solid-

phase synthesis conditions. It was not a consequence of introducing the SAACQ ligand or the SAACQ-Re complex.

The affinity of **5** and **6** for the formyl peptide receptor was determined by flow cytometry using fluorescein labeled fNLFNTK as the reference ligand. Compounds **5** and **6** showed K_d values of 11 ± 3 nm and 27 ± 13 nm which is comparable to that for the parent targeting agent and for fMLF(SAAC)G and fMLF[(SAAC-$Re(CO)_3)^+$]G.

With the approach reported here, it is possible to prepare virtually any small peptide-conjugate with a synthon that can be used as a fluorescent probe and as a tracer for radioimaging studies. The ability to directly correlate in vitro and in vivo imaging studies goes a long way towards bridging the gap between work in isolated cells and studies carried out in living models. This will have a significant impact on biochemical research and on the radiopharmaceutical and pharmaceutical development processes, where, particularly in the latter arena, molecular imaging, both in vitro and in vivo, is playing an increasingly important role.

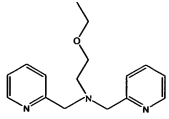
Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

All HPLC experiments were performed on a Varian Prostar HPLC equipped with Autosampler (Model 410), UV-visible detector (Model 345), NaI radiometic detector, and Prostar Pumps (model 210). The preparation of the 0.05 M Triethylammonium phosphate pH 2.25 HPLC solvent was performed by adding 7 mL of triethylamine to 500 mL of H_2O. This was followed by the addition of 4 mL of phosphoric acid to reach the desired 2.25 pH. The solution was diluted to 1000 mL with H_2O and filtered through a 0.22 μm cellulose filter into a 1 liter HPLC bottle. The solution was sonicated for 10 minutes to degas.

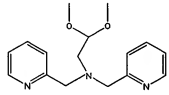
Technetium-99m was used as a $Na^{99m}TcO_4$ solution in saline, as a commercial $^{99}Mo/^{99m}Tc$ generator eluant (Cardinal Health). Technetium-99m (^{99m}Tc) is a γ emitter (141 keV) with a half-life of 6h. The ^{99m}Tc-containing solutions were always kept behind sufficient lead shielding. The use of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared from commercially available Isolink™ kits (Mallinckrodt). The Tc-99m-complexes were prepared and injected as a 10% ethanol / saline solutions.

[N-ethyl-ethoxy-dipyridine-2-methylamine] (1).



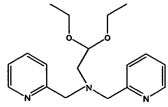
Placed 2-di-(picoline)amine (0.50 g, 2.51 mmol) and 1-bromoethyl-ethoxy(0.420 g, 2.76 mmol) in a 100 mL pressure tube with a stir bar. The solids were dissolved in 2 mL of dried DMF. Potassium carbonate (0.05 g, 0.362 mmol) and NEt_3 (1 mL) were added to the solution. The solution was heated at 125 °C for 4 hrs. and then vacuumed down to residue. The residue was passed through a silca gel column using 2% methanol / methylene chloride as the solvents. The product was eluted as a yellow oil (0.568 g, 83.3 %). ^1H NMR ($CDCl_3$), 300 MHz): 1.12 (t, 3H), 2.79 (t, H), 2.84 (s, 2H), 2.91 (s, 2H), 3.39 (q, H), 3.52 (t, H), 3.87 (s, 2H), 5.24 (s, H), 7.11 (t, 2H), 7.54 (m, 2H), 7.60 (m, H), 7.97 (s, H), 8.47 (d, 2H). GCMS = M.W. 273. Calc. M.W. = 272.

[N-ethyl-dimethoxy-dipyridine-2-methylamine] (2).



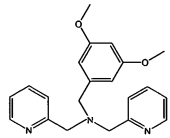
The dipyridine-2-methylamine (0.50 g, 2.51 mmol) was placed in a 15 mL pressure tube equipped with a stirrer. The solution was dissolved in 3 mL of DMF, 2 mL of triethylamine, followed by addition of potassium carbonate (0.10 g, 0.72 mmol), and the 2-bromo-1, 1-dimethoxy-ethane (0.47 g, 2.76 mmol). The solution was heated at 110 °C for 1 hrs. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-10% methanol / methylene chloride as the solvents, yielding 0.25 g, 34.7% yield. ^1H NMR (($CDCl_3$), 300 MHz): 2.77 (d, 2H), 3.28 (s, 6H), 3.92 (s, 4H), 4.53 (t, H), 7.12 (t, 2H), 7.55 (d, 2H), 7.65 (m, 2H), 8.51 (d, 2H), GC/MS = 288 (M+1).

[N-ethyl-diethoxy-dipyridine-2-methylamine] (3).



The dipyridine-2-methylamine (0.50 g, 2.51 mmol) was placed in a 15 mL pressure tube equipped with a stirrer. The solution was dissolved in 3 mL of DMF, 2 mL of triethylamine, followed by addition of potassium carbonate (0.10 g, 0.72 mmol), and the 2-bromo-1, 1-diethoxy-ethane (0.54 g, 2.76 mmol). The solution was heated at 130 °C for 1 hrs. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-10% methanol / methylene chloride as the solvents, yielding 0.51 g, 64.6% yield. ^{1}H NMR ($(CDCl_3)$, 300 MHz): 1.14 (t, 6H), 2.77 (d, 2H), 3.44 (m, 2H), 3.59 (m, 2H), 3.92 (s, 4H), 4.63 (t, H), 7.11 (dd, 2H), 7.56 (d, 2H), 7.64 (m, 2H), 8.48 (d, 2H), GC/MS = 316.

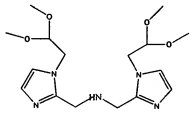
[N-3, 5-dimethoxybenzyl -dipyridine-2-methylamine] (4).



Placed 2-di-(picoline)amine (0.50 g, 2.51 mmol) and 3, 5-dimethoxybenzyl bromide(0.698 g, 3.02 mmol) in a 100 mL pressure tube with a stir bar. The solids were dissolved in 2 mL of dried DMF. Potassium carbonate (0.05 g, 0.362 mmol) and NEt_3

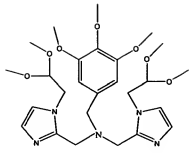
(1mL) were added to the solution. The solution was heated at 125 °C for 1.5 hrs. and then vacuumed down to residue. The residue was passed through a silca gel column using 2% methanol / methylene chloride as the solvents. The product was eluted as a yellow oil (0.50 g, 57.1 %). ^{1}H NMR ($CDCl_3$), 300 MHz): 2.83 (s, 2H), 2.89 (s, 2H), 3.61 (s, 2H), 3.74 (s, 3H), 3.78 (s, 3H), 6.31 (t, H), 6.58 (d, 2H), 7.09 (t, 2H), 7.59 (m, 4H), 8.47 (d, 2H). GCMS = M.W. 351. Calc. M.W. = 349.

[N-{ethyl-2-dimethoxy}-2-imidazolecarboxaldehyde] (5).



The 2-imidazolecarboxaldehyde (2.0 g, 0.021 mol) was placed in a 15 mL pressure tube equipped with a stirrer under argon. The solution was dissolved in 2 mL of DMF, followed by addition of potassium carbonate (0.50 g, 3.6 mmol), and bromoacetaldehyde dimethyl acetal (03.87 g, 0.023 mmol). The solution was heated at 120 °C for 20 hrs. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-10% methanol / methylene chloride as the solvents, yielding 1.15 g, 30.1% yield. ^{1}H NMR ($CDCl_3$), 300 MHz): 3.37 (s, 6H), 4.47 (m, 2H), 7.20 (s, H), 7.25 (s, H), 9.78 (s, H).

[N-{ethyl-2-dimethoxy}-2-methyl-imidazole-3,4,5-trimethoxy-benzylamine] (6).



Placed 3,4,5-trimethoxy-benzylamine (0.054 g, 0.027 mol) in a 100 mL round-bottom flask equipped with a stirrer under nitrogen. The liquid was dissolved in 8 mL of dichloroethane, followed by addition of [N-{ethyl-2-dimethoxy}-2-imidazolecarboxaldehyde] (0.10 g, 0.054 mmol) and sodium triacetoxyborohydride (0.127 g, 0.059 mmol). The solution was stirred at room temperature for 18 hours. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-5% methanol / methylene chloride as the solvents, yielding 0.124 g, 85.5% yield. ^{1}H NMR ($CDCl_3$), 300 MHz): 3.21 (s, 12H), 3.54 (s, 2H), 3.72 (s, 4H), 3.80 (s, 3H), 3.82 (s, 6H), 3.87 (d, 4H), 4.20 (t, 2H), 6.43 (s, 2H),, 6.92 (d, 2H). GCMS = 535 (M+1).

Technetium-99m labeling:

The technetium labeling was accomplished using the Tc(I)-tricarbonyl methods. The $Tc(I)(CO)_3^+$ core was readily formed using the Isolink™ kit (Mallinkrodt). The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ starting material was formed by adding 1 mL of TcO_4^- in saline to an Isolink™ kit. The solution was heated at 100°C for 30 minutes, followed by the addition of 120 µl of 1N HCl to neutralize the solution. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (200 µl) was added to the appropriate derivative in 0.2 mL (1mg / mL) of methanol and heated at 80° C for 1 hour.

Analysis of the reaction products using C18 HPLC, showed >60% RCP for all complexes. The HPLC analysis was performed using a Vydac C18 column, 25cm x 4.6mm column (5μm pore size), equipped with a 2 cm guard column. Solvent A was 0.05 M triethylammonium phosphate buffer pH 2.5 and solvent B was methanol. The method employed a gradient run over 30 minutes at a flow rate of 1ml / minute. The gradient ramped from 5-100% B from 3-20 minutes.

Animal Studies:

The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. The vertebrate animals in this research project were used to investigate the biodistribution and pharmacokinetics of the rotenone derivatives and determine uptake in the heart. Rats (Sprague Dawley, male, at 80-100 grams each) were used for the whole body biodistribution studies. The Tc-complexes, as well as Cardiolite™, were evaluated at three time points; 5, 30, and 120 minutes, with five animals per time point. In order to provide accurate statistics in the clearance rate measurements and to account for intra-species variation it was necessary to use this number of animals. The product was diluted to ~10μCi / 100 μl using freshly prepared 10% ethanol / saline (0.9%) solution. The rats were injected via a lateral tail vein with a volume of 0.1 mL. The rats were sacrificed by decapitation, with immediate blood collection at the desired time points. Whole body biodistributions were performed on the animals immediately following decapitation, organ and tissue samples were taken and washed of excess blood, blotted dry and weighed. Radioactivity was assayed using automated NaI well counter. All

tissue samples were counted together along with an aliquot of the injected dose so that % injected dose and % injected dose per gram of tissue could be calculated. The data are reported as %ID/g.

Biodistribution Results:

The purpose of this study was to investigate the biological potential of a novel series of Tc-99m-dipyridine derivatives as heart blood flow imaging agents. The evaluation was based on the uptake and retention of the Tc-99m-dipyridine derivatives in rats. These novel complexes, as well as Cardiolite™, were evaluated at three time points; 5, 30, and 120 minutes, five animals per time point. The results are reported in **Tables 1** and **2**, as well as depicted graphically in **Figure 5.**

Table 1. *Biodistribution summary (%ID/g ± SEMs) of the four Tc-dipyridine derivatives.*

	Tc-DP-ethyldimethoxy (Tc-2)	Tc-DP-ethyldiethoxy (Tc-3)	Tc-DP-3,5-dimethoxybenzyl (Tc-4)	Tc-DI-3,4,5-trimethoxybenzyl (Tc-6)
BLOOD 5'	0.16 (0.027)	0.11 (0.014)	0.93 (0.004)	0.43 (0.133)
BLOOD 30'	0.11 (0.006)	0.07 (0.003)	0.03 (0.003)	0.05 (0.003)
BLOOD 120'	0.06 (0.004)	0.03 (0.001)	0.02 (0.001)	0.02 (0.002)
HEART 5'	0.56 (0.121)	0.97 (0.275)	0.84 (0.075)	1.49 (0.331)
HEART 30'	0.80 (0.069)	1.49 (0.173)	0.78 (0.049)	2.34 (0.122)
HEART 120'	0.78 (0.047)	1.56 (0.153)	0.76 (0.083)	1.81 (0.064)
LUNG 5'	0.33 (0.044)	0.33 (0.070)	0.27 (0.032)	0.63 (0.130)
LUNG 30'	0.29 (0.026)	0.44 (0.067)	0.17 (0.013)	0.57 (0.045)
LUNG 120'	0.23 (0.028)	0.32 (0.027)	0.11 (0.017)	0.42 (0.065)
LIVER 5'	0.80 (0.208)	0.44 (0.102)	0.64 (0.052)	1.90 (0.483)
LIVER 30'	0.40 (0.027)	0.19 (0.009)	0.17 (0.017)	0.65 (0.039)
LIVER 120'	0.20 (0.023)	0.12 (0.009)	0.09 (0.005)	0.28 (0.019)
KIDNEY 5'	5.51 (1.344)	3.54 (0.917)	7.53 (0.805)	4.40 (0.813)
KIDNEY 30'	1.99 (0.364)	1.34 (0.084)	4.57 (0.365)	4.61 (0.199)
KIDNEY 120'	0.58 (0.026)	0.87 (0.057)	1.11 (0.064)	3.44 (0.256)

*TABLE 2. Selected ratios (± SEMs) of target organs.**

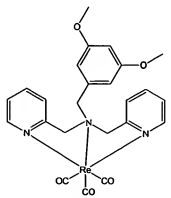
Ratios	Tc-DP-ethyl-dimethoxy (Tc-2)	Tc-DP-ethyl-diethoxy (Tc-3)	Tc-DP-3,5-dimethoxy-benzyl (Tc-4)	Tc-DI-3,4,5-trimeth xy-benzyl (Tc-6)	Cardiolite™**
HT/BL 5'	3.45	8.18	8.99	5.07	8.16 (0.524)
HT/BL 30'	7.12	22.4	23.1	51.8	
HT/BL 120'	13.9	44.0	52.0	92.9	
HT/LIV 5'	0.78	2.13	1.38	0.89	4.13 (0.893)
HT/LIV 30'	2.03	7.72	4.96	3.65	2.5 †
HT/LIV 120'	4.08	12.3	8.73	6.72	
HT/LU 5'	1.64	2.81	3.27	2.56	2.19 (0.065)
HT/LU 30'	2.74	3.55	4.57	4.22	5.6 †
HT/LU 120'	3.56	5.03	7.93	4.54	

*BL = blood, HT = heart, LU = lung, LIV = liver.
** Cardiolite™ was evaluated at 5 minutes in 90 gram rats.

†**The other ratios are from** Boschi, A. *et al.* Synthesis and Biological Evaluation of Monocationic Asymmetric 99mTc-Nitride Heterocomplexes Showing High Heart Uptake and Improved Imaging Properties. J. Nucl. Med. (2003) 44: 806-814.

Rhenium Chemistry:

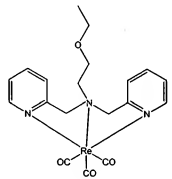
[Re(CO)$_3$(N-3,5-dimethoxybenzyl-dipyridine-2-methylamine)](7).



The [NEt$_4$]$_2$[Re(CO)$_3$(H$_2$O)$_3$] (0.015 g, 0.019 mmol) and 2-di(picoline)amine-*N*-3, 5-dimethoxybenzyl (KM08-121) (0.0068 g, 0.019 mmol) were placed in a 100 mL

pressure tube with a stirr bar. The solids were dissolved in 5 mL of methanol. The solution was heated at 130 °C for 3 hrs. The solution was vacuumed down to residue. The residue was passed through a silca gel column using 10% methanol / methylene chloride as the solvents. The product eluted as the rhenium complex (11.3 mg, 91.5 %). ^{1}H NMR ($CDCl_3$), 300 MHz): 1.17 (s, H), 1.56 (s, 3H), 3.47 (d, H), 3.87 (s, 3H), 4.64 (m, 2H), 5.73 (d, 2H), 6.59 (t, H), 6.75 (d, H), 7.16 (t, 2H), 7.31 (m, H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H). LC/MS = M.W. 620. Calc. M.W. = 619.

[$Re(CO)_3$(N-ethyl-ethoxy-dipyridine-2-methylamine)] (8).



The $[NEt_4]_2[Re(CO)_3(H_2O)_3]$ (0.04 g, 0.052 mmol) and 2-di(picoline)amine-*N*-ethyl ethoxy (KM08-131) (0.014 g, 0.052 mmol) were placed in a 100 mL pressure tube with a stirr bar. The solids were dissolved in 5 mL of methanol. The solution was heated at 130 °C for 2 hrs. The solution was vacuumed down to residue. The residue was passed through a silca gel column using 10% methanol / methylene chloride as the solvents. The product eluted as the rhenium complex (8 mg, 28.6 %). ^{1}H NMR ($CDCl_3$), 300 MHz): 1.25 (t, 3H), 3.72 (d, 2H), 3.97 (t, 2H), 4.05 (t, 2H), 4.55 (d, 2H), 6.10 (d, 2H), 7.18 (t, 2H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H). LC/MS = M.W. 542.3 Calc. M.W. = 542.2.